

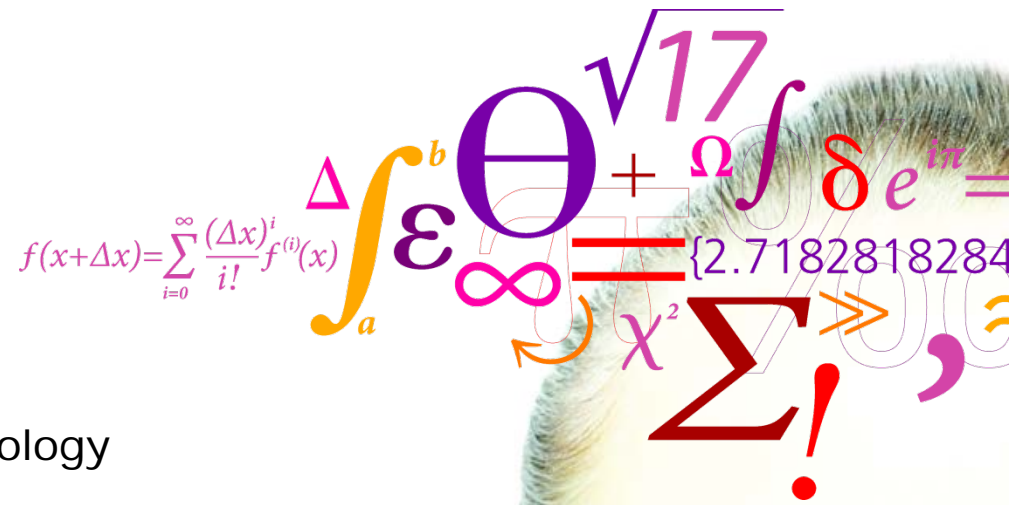
# Update from the EURL-AR 2019

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 Technical University of Denmark

DTU Food  
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Annual EURL AR workshop  
 29 April 2020  
 DTU, Kgs. Lyngby, Denmark



# To ensure availability and use of high quality methods / high quality performance by NRLs

Sub-activity 1.1 (Providing NRLs with details and guidance on laboratory methods)

- ESBL protocols slightly updated in relation to 1.2 (Vers. 7)
  - description of and logic behind the prolonged period for the microbiological analysis (48-96h) conflicted with the concept of meat samples having an expiration date as indicated on the label of the meat samples
- The comment field was updated with the results of the 96h validation experiments performed at the EURL-AR in 2018



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LABORATORY PROTOCOL

Isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* from caecal samples

December 2019  
Version 7

Version 7 was reviewed and updated by: Rene S. Hendriksen and Valeria Bortolota  
Authors of the document: Henrik Hasman, Yvonne Agesta, Rene Hendriksen, Lisa M. Carisco (DTU Food) and Beatrix Guerra-Roman (external expert)

HISTORY OF CHANGES				
Version	Sections changed	Description of change	Date	Author
7	Comment to tables 1.2, 1.3 and 1.4	The maximum time for isolation and test results of the 96h validation experiments mentioned in the table is 96h.	08 Dec 2019	Rene Hendriksen, Valeria Bortolota
6	1.1, 1.1.1, 1.1.2, 1.1.3, 1.1.4, 1.1.5, 1.1.6, 1.1.7, 1.1.8, 1.1.9, 1.1.10, 1.1.11, 1.1.12, 1.1.13, 1.1.14, 1.1.15, 1.1.16, 1.1.17, 1.1.18, 1.1.19, 1.1.20, 1.1.21, 1.1.22, 1.1.23, 1.1.24, 1.1.25, 1.1.26, 1.1.27, 1.1.28, 1.1.29, 1.1.30, 1.1.31, 1.1.32, 1.1.33, 1.1.34, 1.1.35, 1.1.36, 1.1.37, 1.1.38, 1.1.39, 1.1.40, 1.1.41, 1.1.42, 1.1.43, 1.1.44, 1.1.45, 1.1.46, 1.1.47, 1.1.48, 1.1.49, 1.1.50, 1.1.51, 1.1.52, 1.1.53, 1.1.54, 1.1.55, 1.1.56, 1.1.57, 1.1.58, 1.1.59, 1.1.60, 1.1.61, 1.1.62, 1.1.63, 1.1.64, 1.1.65, 1.1.66, 1.1.67, 1.1.68, 1.1.69, 1.1.70, 1.1.71, 1.1.72, 1.1.73, 1.1.74, 1.1.75, 1.1.76, 1.1.77, 1.1.78, 1.1.79, 1.1.80, 1.1.81, 1.1.82, 1.1.83, 1.1.84, 1.1.85, 1.1.86, 1.1.87, 1.1.88, 1.1.89, 1.1.90, 1.1.91, 1.1.92, 1.1.93, 1.1.94, 1.1.95, 1.1.96, 1.1.97, 1.1.98, 1.1.99, 1.1.100	Update of the protocol to reflect the results of the 96h validation experiments performed at the EURL-AR in 2018.	08 Dec 2019	Rene Hendriksen, Valeria Bortolota
5	1.1 and 1.2	Update of the protocol to reflect the results of the 96h validation experiments performed at the EURL-AR in 2017.	08 Dec 2017	Rene Hendriksen, Valeria Bortolota
4	1.1 and 1.2	Update of the protocol to reflect the results of the 96h validation experiments performed at the EURL-AR in 2015.	08 Dec 2015	Rene Hendriksen, Valeria Bortolota
3	All through the document	Update of the protocol to reflect the results of the 96h validation experiments performed at the EURL-AR in 2014.	08 Dec 2014	Authors
2	All through the document	Update of the protocol to reflect the results of the 96h validation experiments performed at the EURL-AR in 2013.	21 Nov 2013	Authors
1	New document	Initial version of the protocol.	21 Nov 2013	Authors

# To ensure availability and use of high quality methods / high quality performance by NRLs

Sub-activity 1.1 (Providing NRLs with details and guidance on laboratory methods)

- Survey to determine how many laboratories that perform MRSA detection and ESBL quantification in caecal and/or meat samples and which methods are applied
  - Analyses are not mandatory and indeed only a relatively low number of laboratories perform or had performed them
- 24 laboratories that replied to the survey, 11 and six reported to have performed MRSA detection and ESBL quantification, respectively
  - Mainly as ad hoc projects and not routinely
  - 7 out of 11 laboratories performing MRSA detection according to the EURL-AR protocol
  - 3 out of 6 laboratories performing ESBL quantification according to the EURL-AR protocol

# To ensure availability and use of high quality methods / high quality performance by NRLs

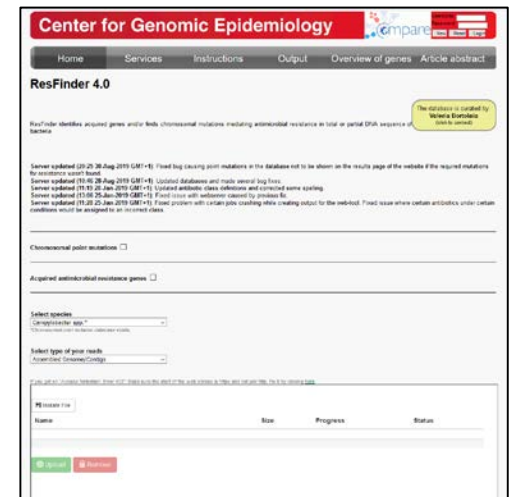
Sub-activity 1.1 (Providing NRLs with details and guidance on laboratory methods)

- The finalized MIC survey containing 45 images of MIC panels assessed by the NRLs showed a worrisome picture that deviations from the expected results were mainly due to discordant reading by the NRLs
- In 2019, continued to advise the NRLs when approached on how to interpret both phenotypic antimicrobial susceptibility tests but also the genetic mechanisms responsible for the phenotypic resistance
  - This was especially emphasized when elaborating the confirmatory test results improving the knowledge on methodologies for detection of ESBL, AmpC and carbapenemase-producing E. coli and Salmonella, and associated genetic mechanisms related to phenotypic resistance

# To ensure availability and use of high quality methods / high quality performance by NRLs

Sub-activity 1.1 (Providing NRLs with details and guidance on laboratory methods)

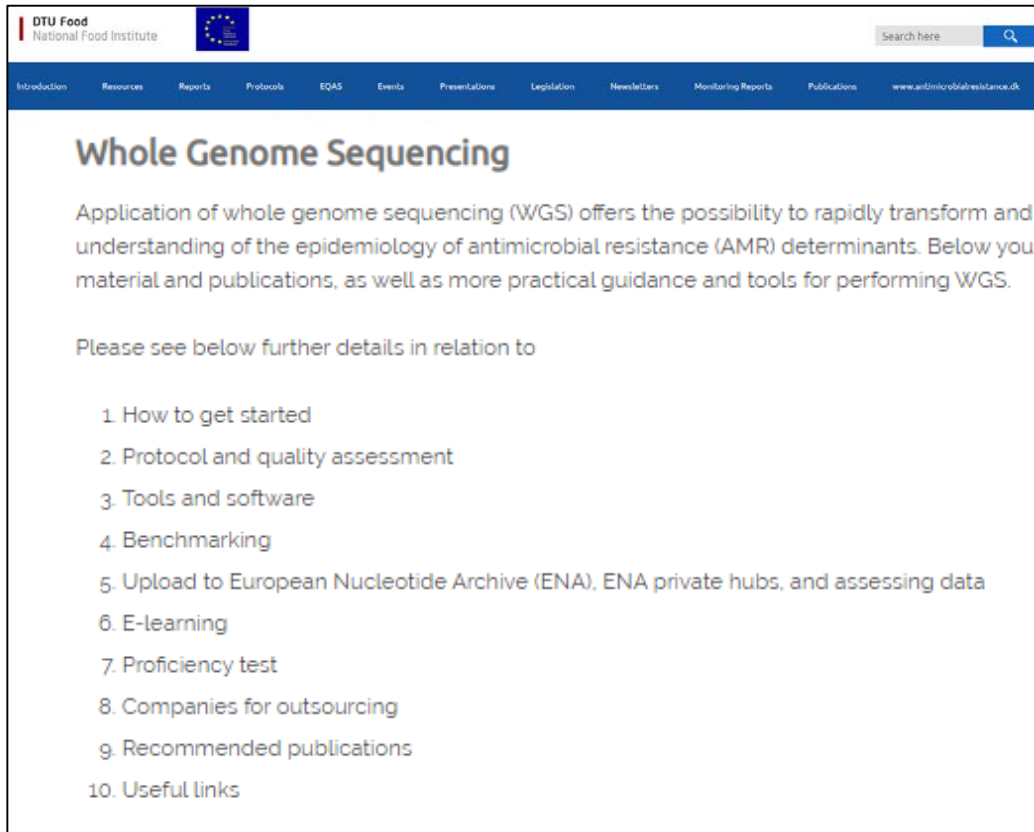
- Continue the development of the Bacterial Analysis Pipeline (BAP) that enables users to detect genetic determinants of resistance (including chromosomal point mutations and acquired antimicrobial resistance genes) along with other molecular traits in genomes submitted as bulk volumes
- In 2019, ResFinder 4.0 available and include 2,690 AMR genes and 266 resistance mediating mutations in 39 selected genes from *Escherichia coli*, *Salmonella* sp., *Campylobacter* sp., *Enterococcus faecium* and *Enterococcus faecalis* and *Staphylococcus aureus*



# To ensure availability and use of high quality methods / high quality performance by NRLs

Sub-activity 1.1 (Providing NRLs with details and guidance on laboratory methods)

- Harmonization of WGS in order to obtain comparable data across EU



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## Whole Genome Sequencing

Application of whole genome sequencing (WGS) offers the possibility to rapidly transform and understanding of the epidemiology of antimicrobial resistance (AMR) determinants. Below you material and publications, as well as more practical guidance and tools for performing WGS.

Please see below further details in relation to

1. How to get started
2. Protocol and quality assessment
3. Tools and software
4. Benchmarking
5. Upload to European Nucleotide Archive (ENA), ENA private hubs, and assessing data
6. E-learning
7. Proficiency test
8. Companies for outsourcing
9. Recommended publications
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EUROPEAN UNION

PROTOCOL FOR WHOLE GENOME SEQUENCING AND BIOINFORMATIC ANALYSIS OF BACTERIAL ISOLATES RELATED TO THE EU MONITORING OF ANTIMICROBIAL RESISTANCE

AUTHORED BY THE EURL-AR

1<sup>st</sup> VERSION - JANUARY 2020

Version	Sections changed	HISTORY OF CHANGES		
		Description of change	Date	Approval
1	All through the document	Minor modifications based on comments from the EURL Working group of NGS	10 Jan 2020	The EURL Working Group of NGS
Draft	New draft document	-	06 Dec 2019	Authors

1

# To ensure availability and use of high quality methods / high quality performance by NRLs

Sub-activity 1.2 (Providing reference materials to NRLs)

- Continued to update the strain collection at the EURL-AR which is available for the NRLs in the EURL-AR network
  - Provision of various reference strains or relevant strains containing specific encoding resistance genes
    - Sent at least 18 parcels containing quality control strains



# To ensure availability and use of high quality methods / high quality performance by NRLs

Sub-activity 1.3 (Coordination and organization of inter-laboratory comparative testing)

- In 2019, the EURL decided to invest an in-kind contribution of 88.500 EUR to develop new EQAS databases for the five bacterial species included the EU EQAS scheme
- The new databases was available for submission and evaluation of the EQAS results as of 2019
- The development continued in 2020 with a new database for the Matrix EQAS component
- The new databases are more sustainable since they are less vulnerable to staff replacement and furthermore both design and output accessibility have been improved



# To ensure availability and use of high quality methods / high quality performance by NRLs

Sub-activity 1.3 (Coordination and organization of inter-laboratory comparative testing)

- Trial 1 (October)
  - Salmonella
  - Campylobacter
  - Genotypic characterization also including detection of ESBL, carbapenem, and AmpC genes
  
- Trial 2 (June)
  - Escherichia coli
  - Enterococci
  - Staphylococci incl. MRSA
  
- Trial 3 (September)
  - Matrix EQAS, including qualitative detection of ESBL and AmpC producing E. coli from a matrix of caecal and food samples (cattle swine, and chicken/ beef pork, and chicken meat)



- Published three reports all related to the EQASs in 2018

# To ensure availability and use of high quality methods / high quality performance by NRLs

Sub-activity 1.3 (Coordination and organization of inter-laboratory comparative testing)

- The EURL-AR initiated the preparation of an inter-laboratory comparative test scheme and system for measuring the quality of genomes and in silico antibiograms produced by WGS
- The EURL received a grant from the UK Fleming Fund to develop the EQAS database for the in silico antibiograms reporting for the three bacterial species included the genomic EQAS scheme
- The genomic EQAS has been prepared in 2019 and an invitation to sign up was distributed to the EURL-AR network in December 2019
- Expected to be ready for launch in September 2020 – delayed due to Covid-19

# To provide scientific and technical assistance to NRLs

## Sub-activity 2.1 (Coordinating practical arrangements and informing NRLs)

- The EURL-AR maintained and continuously updated the full list of contact persons from all NRLs
  - Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France (NRL split in 3 institutes), Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania (NRL split in 2 institutes), Slovakia, Slovenia, Spain (NRL split in 3 institutes), Sweden, United Kingdom (NRL split in 2 institutes)
  - Iceland, Norway and Switzerland have been invited to participate in the network as non-EU countries
  - North Macedonia, Serbia and Turkey have been invited to the network activities as candidate countries
  - Collaborating with colleagues in Albania, Montenegro, Bosnia-Herzegovina, and Kosovo

# To provide scientific and technical assistance to NRLs

## Sub-activity 2.2 (Conducting training courses for NRLs)

- A training course was held from September 24-27, 2019
- The theme for the training course was in concordance with the EURL Work Group on Next Generation Sequencing and titled “Enhancing WGS capacity for AMR surveillance in the EURL-AR network”
- The course aimed at providing participants with the necessary knowledge to i) perform antimicrobial resistance (AMR) surveillance using WGS, and ii) critically assess advantages/disadvantages compared to phenotype-based AMR surveillance.
  - 37 participants and 14 trainers from 31 countries
  - Included wet-lab training sessions and bioinformatics exercises
- Two observers from WHO and FAO participated to the training course

# To provide scientific and technical assistance to NRLs

Sub-activity 2.3 (Missions and specific assistance to individual laboratories)

- The EURL-AR performed a site-visit mission to a National Reference Laboratory for Antimicrobial Resistance
- The first candidate country to visit - 19<sup>th</sup> to 20<sup>th</sup> November 2019
- The aim of the visit was to review the results of EURL-AR EQAS
  - assistance in relation to general AST method guidelines, recommendations, quality control procedures in AST and management of reference strains

# To provide scientific and technical assistance to NRLs

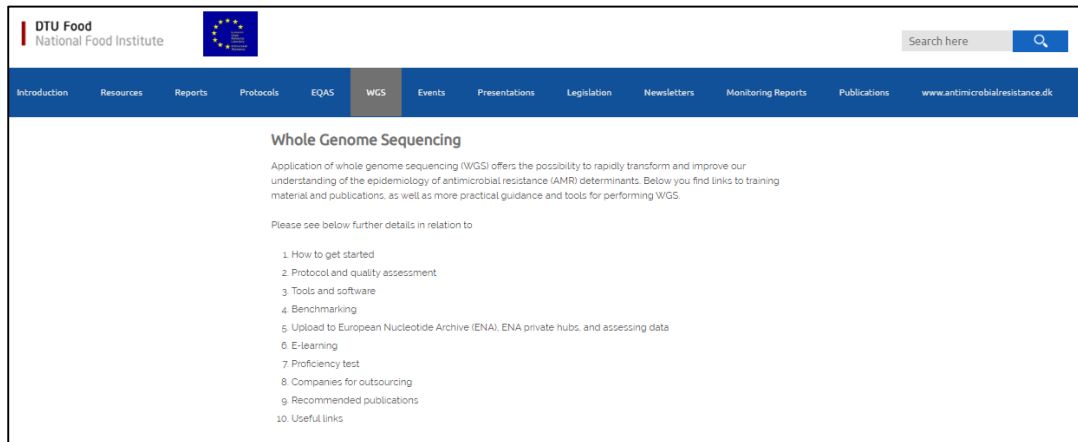
Sub-activity 2.4 (Coordination and organization workshops among the NRLs)

- The 13<sup>th</sup> EURL-AR workshop was held during April 25-26, 2019
  - All member states were represented at the workshop except for Cyprus, Malta and Finland
  - Participating non-EU countries were Albania, Iceland, North Macedonia, Norway, and Switzerland
  - Three researchers from Ghana (funded by the Fleming Fund) and two from Thailand (funded by FAO/IAEA) participated as observers as did also a representative from Federation of Veterinarians of Europe (FVE).

# To provide scientific and technical assistance to NRLs

Sub-activity 2.5 (Providing information on relevant research activities to NRLs)

- The <https://www.eurl-ar.eu/> contains all relevant information on resources available from the EURL-AR
  - google analytics state that the web site had 412 unique visitors per month



- At least 22 emails have been disseminated to the NRLs including relevant information from the EC, Directorate F, EFSA, ECDC, EMA, and WHO
- The EURL-AR has posted one newsletter, no 13 in 2019

# To provide scientific and technical assistance to NRLs

## Sub-activity 2.6 (Providing E-learning)

- In 2019, the EURL-AR updated the current version of the lecture on colistin in the AMR e-learning course
  - Includes the new *mcr* gene variants discovered since the first lecture was posted
  - reached by more than 114,060 and 28,048 participants enrolled from >150 countries around the world

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## Antimicrobial resistance – theory and methods

**About this course:** The course will cover the topics related to antimicrobial resistance with basic definitions and overview on antimicrobials their use and the emergence and spread of resistance. The course will guide you through the concepts and the importance of resistance spread and dissemination and how that happens. It will show you how bacteria become resistant and which mechanisms they might

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**Who is this class for:** This course is for you if you are interested in getting to know more about antimicrobials and antimicrobial resistance in bacteria. We aim at having a broad scope and international reach in different sectors. So this course us for you whether you are an undergraduate or graduate student, a researcher, medical or veterinary related professional, technical staff or simply interested in the subject

**Created by:** Technical University of Denmark (DTU)

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**Enroll**  
Started Oct 30



# To provide scientific and technical assistance to NRLs

## Sub-activity 2.7 (Confirmatory testing)

- In 2019, the EURL-AR has received in total 384 strains of Salmonella and E. coli from 31 European countries
- A total of 8,376 MIC determinations were conducted – some genomes still pending due to C19
- Four countries provided their own sequence data for analysis at the EURL-AR
- The produced data will be provided to each MS and EFSA and genomes submitted to ENA to a private repository with restricted access for two years
- All updated data will be submitted to the scientific EFSA report on “The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2018”

# To provide scientific and technical assistance to the European Commission and other organisations

Sub-activity 3.1 (Providing scientific and technical assistance to the EU Commission)

- EURL continued the contribution to drafting the technical specification “Technical specifications on harmonised monitoring of resistance in zoonotic and indicator bacteria from food-producing animals and food”
- Provided scientific and technical assistance to EC in relation to the draft Decision

Non-paper  
in view of a possible EC decision on the  
monitoring and reporting of  
antimicrobial resistance in zoonotic and  
commensal bacteria and repealing  
Commission Implementing Decision  
2013/652/EU

# To provide scientific and technical assistance to the European Commission and other organisations

Sub-activity 3.2 (Provide scientific and technical assistance to others than EU Commission)

- SEQAFRICA – setting up WGS centres in four countries in Africa
  - Bioinformatic training
  - Hardware acquisition
  - Provision of consumables
  - PT
  - Projects and ref. test of AMR isolates
    - Target: 15,000 strains
- EQASIA / EQAFRICA – providing EQA, eight WHO GLASS pathogens to Asia and Africa



# Reagents and Reference collections

Sub-activity 4.1 (Establish and maintain reference material and resources)

- The strain and primer collections at the EURL-AR are available for the NRLs in the EURL-AR network
  - In 2019, the collection was only maintained due the lack of new important identified resistance genes – in the process of retrieving new mcr variant – now up to mcr-10

# Thank you for your attention



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